Chapter 2

Basic Gas Chromatography

2.1 Introduction and History

This chapter will focus on the components and operation of basic gas chromatography (GC). The general field of chromatography dates back to 1903 with the work of Russian scientist Mikhail Tswett, who separated plant pigments using liquid chromatography. Fritz Prior, as part of his graduate work, developed solid-state gas chromatography in 1947. Modern gas chromatography is generally considered to have been invented by Martin and James in 1952. A review of the history of gas chromatography can be found in Bartle and Myers (2002). Since 1952, gas chromatography has advanced from using solid spheres to act as the stationary phase (gas-solid chromatography) to using liquid coated resins as the stationary phase, and finally to using covalently-bonded stationary phases attached to wall of a capillary column (gas-liquid chromatography). Components of the actual chromatograph have also advanced from many manual parts such as rotary gas flow regulators being updated to electronic flow or mass flow controllers, resin packed column have been replaced with fused silica capillary columns, and manual injection and control of the instrument has been replaced with automated injection and computer control. Most notably is the diversity of detectors utilized today with GC, especially the ability to connect capillary column GCs with mass spectrometers for confirmatory analysis. Additionally, in the past, analyses of a set of samples could take days to complete and required the constant attention of an analyst, but today with the help of computers, a set of samples and standards can be started on the instrument and the scientist can return later with all of the samples analyzed and the data processed. Typical automatic sampler units can hold up to 100 samples. These improvements have greatly increased the capabilities of laboratories and advanced scientific endeavors but in many cases have decreased the analyst's knowledge of the chromatographic system. But such is the price of advancement and economics. In this chapter, we will discuss the types of samples and analytes that can be analyzed by GC, the components of the GC and their operation, the variety of detectors available today for use with GC, and examples of specific analyses.

2.2 Types of Samples and Sample Introduction

A basic GC of reasonable quality costs from 30,000 to 50,000 US dollars today depending on the detectors that are purchased with the GC, although more inexpensive models can be purchased for limited routine analysis. A capillary column gas chromatography-mass spectrometer (GC-MS, quadrupole) will cost slightly less than $100,000. With this relatively high price tag, students sometimes trust the results as unquestionably accurate. Reality could not be farther from this belief. Every step, including extraction of the analytes from the sample matrix, conducting serial dilutions, injection into the GC, and identification of the fragmentation pattern in MS is prone to errors. From experience, sample extraction can be the most difficult and is the source of considerable error. Samples come in a variety of forms: gaseous, liquid, solid, and biological. In order for the reader to fully appreciate sample preparation for GC analysis the following discussion will present several sample collection, extraction and sample preparation techniques.

2.2.1 Gaseous Samples

Gaseous samples are the easiest samples to analyze. For on-site analysis, a gaseous sample can simply be drawn into a syringe and the sample injected into a sampling valve/loop. Sampling loops are necessary in GC analysis in order to inject a consistent volume of a compressible sample. When a gas sample is taken at atmospheric pressure and injected into a GC inlet, the pressure in the GC will compress the gas in the syringe and not allow all of the sample volume to be injected. A sampling valve and loop consist of a four- or six-port valve that allows the sample to be injected into a fixed-volume (loop of tubing that is at atmospheric pressure. A valve is then turned that transfers all of the gas contained in the sample loop into the GC injection port. For field gaseous samples that need to be transported to the laboratory for analysis, a variety of sampling containers are available including Teflon bags and metal cylinders (referred to as bombs) that can be filled with the sample gas. It should be noted that when these containers are analyzed that they be adjusted back to their field temperature in order to avoid condensation of some gaseous analytes to liquids; this is especially true when industry smoke stack or process gases are being sampled and analyzed. Another possibility for sampling gaseous analytes is a resin tube. To collect a sample a known volume of gas is passed through a glass or metal tube containing a resin that has a strong affinity for the analytes. The analytes adsorb to the resin and after a sufficient volume of gas has passed through the system, each end of the resin tube is capped and transported

back to the lab. In the lab, the resin is extracted with a solvent specific to the analysis and the solvent/analyte solution is injected into the GC. A relatively simple calculation yields the concentration of each analyte in the original gas volume. The obvious advantage of this method is concentration of the gaseous analytes and the improvement of detection limits, as opposed to analyzing the gas by direct injection. The resin tube method is commonly used in the monitoring of solvents in the work place where an industry worker will wear a portable personal pump that takes in atmospheric gases at the same rate as a human would breath under working conditions. At the end of the day, the tube is extracted and analyzed to determine if the worker was exposed to chemicals in excess of workplace limits according to Occupational Safety and Health Association standards.

2.2.2 Liquid Samples

Liquid samples are the next easiest to analyze by GC since they are already in an injectable matrix. Samples from organic synthesis procedures usually have products (analytes) present at high concentrations and are analyzed by direct injection. Unfortunately, relatively few products fit the requirements of GC—that analytes be volatile and thermally stable—so most products are analyzed by HPLC, the subject of Chapter 3. Analytes in aqueous samples are also easy to analyze by GC. Some GC detectors and columns allow the direct injection of aqueous samples if the concentration of the analyte is sufficiently high. The aqueous sample is frequently extracted into an organic solvent using a standard separatory funnel when there is a low concentration of analyte is present in sample or when water could harm the GC column or detector. Usually the aqueous sample is extracted three times with a relatively small volume of organic solvent, the organic extracts are combined, the volume is reduced by evaporation, and the resulting organic extract is injected into the GC. One disadvantage of the organic extraction is the need to purchase expensive and very pure organic solvents (typically priced at approximately $150 for four liters) and the expensive disposal costs of the resulting organic waste solvents. A more automated version of the separatory funnel is a liquid-liquid extractor, but the glassware is relatively expensive and typical extraction times run from 8 to 24 hours. In this extraction setup, the organic solvent is boiled, condensed, and passed through a water vessel where the non-volatile hydrophobic analytes partition into the organic solvent that is constantly recycled into the boiling vessel. The recycled solvent is re-evaporated again, leaving the analytes in the boiling flask, and passes again into the condenser and water column. Figure 2.1 shows a typical liquid-liquid extraction system.

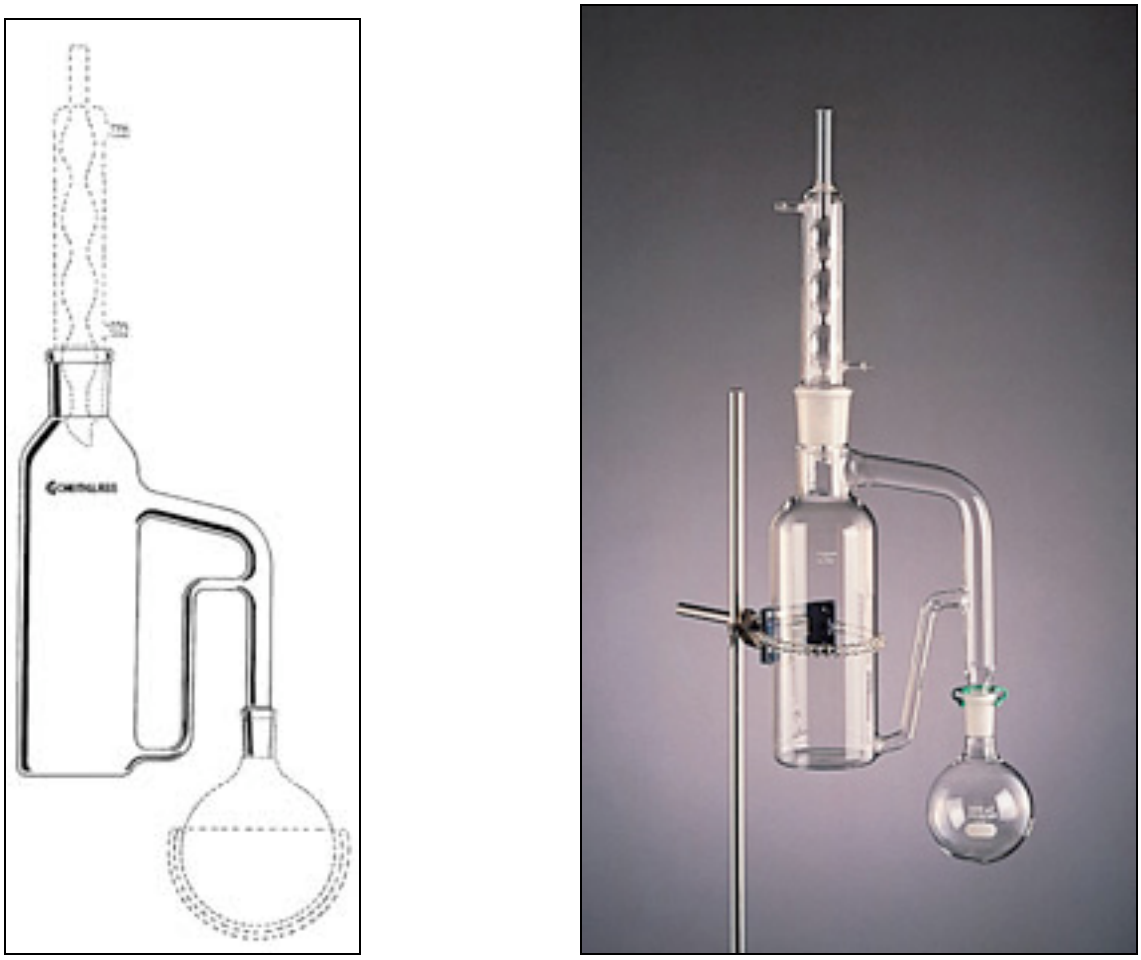


Figure 2.1 A Liquid-Liquid Extractor for Extracting Analytes from Water Samples. Reprinted with permission from VWR Scientific Products International, Chemglass Life Sciences, and Corning.

A relatively easy way to avoid the need for expensive glassware is to use resin packs (SPE; solid phase extraction) that are available from a variety of vendors. In this technique, the water sample is passed through a resin pack (a solvent-resistant tube usually one to a few centimeters in diameter and slightly taller in height). Again, the resin has a high affinity for the analytes. After the passage of the aqueous sample through the packet, the resin is dried by passing ultra-pure gas through it and the adsorbed analytes are removed by passing a small volume of organic solvent (usually a few mL) through the packet. The solvent volume is adjusted to a known volume and injected into the GC.

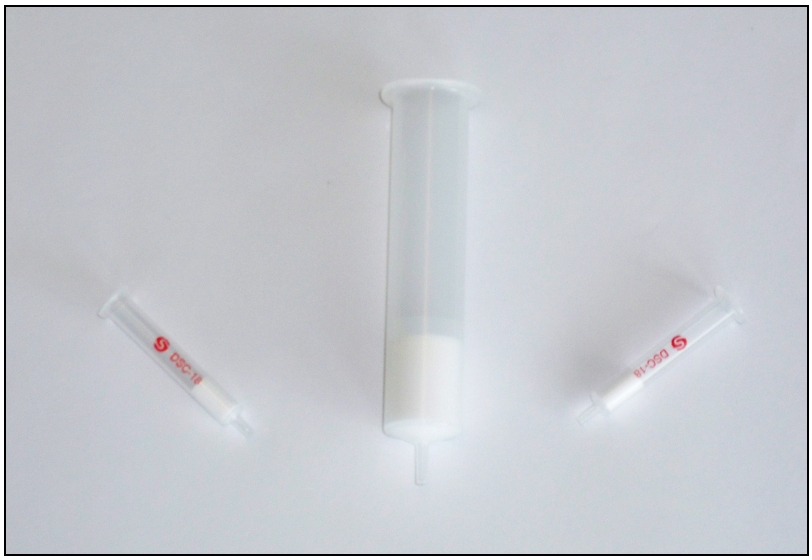


Figure 2.2 Three Resin Packets for Extracting Analytes from Aqueous Samples.

An even more novel way of extracting analytes from water samples is to use Solid Phase Micro Extractors (SPMEs) that consist of a syringe containing a fused silica capillary fiber coated with a chromatography stationary phase with a high affinity for the analytes of interest. The fiber is housed in a metal needle where it can be extended for collecting analytes or for desorption in a GC injection inlet. The SPME needle and fiber are passed through a septum in the sample bottle, either into the gaseous space above the water or directly into the water, the fiber is exposed through the end of the needle and allowed to equilibrate (adsorb the analytes) for typically 10 to 30 minutes while the sample is mixed with a stir bar. After this time most or all of the analytes are transferred to the SPME fiber. The fiber is drawn into the metal needle; the needle is withdrawn from the sample bottle and placed directly into the GC injector. The advantages of this technique are (1) no need for expensive organic extraction solvents, (2) relatively rapid analysis, (3) possibly improved extraction recovery, and (4) significant concentration of the analytes and improvement of detection limits (up to 10 000 to 1 000 000 fold concentrations). Fibers can be reused from 50 to 100 times. The minor disadvantage of the SPME technique is the cost of the apparatus (approximately $600 for three fibers and a holder/injector).

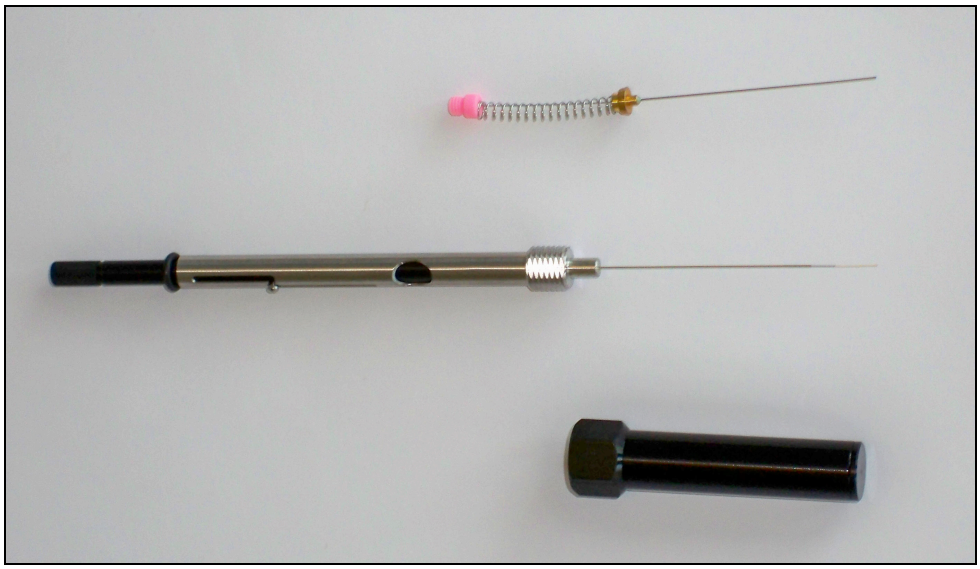


Figure 2.3 A SPME Device with the Microfiber Exposed (middle item). Extra needles are available (top item) since the injector syringe can be reused indefinitely. Needles can be reused from 50 to 100 times depending on the composition of the sample. The bottom item is the needle protection guard and GC injection guide.

Volatile analytes present an additional problem since considerable quantities of the analyte can be lost during sample preparation. Analyses of volatile analytes are best preformed with some type of commercial head-space analyzer or "purge and trap" device where the actual water sample (with no gaseous headspace) is attached to a sample processing unit, a gas is used to transfer the volatile analytes to a resin trap or directly into the GC injector, and after the required purge time the transferred analytes are analyzed by GC.

2.2.3 Soil/Sediment Samples

Soil and sediment samples present considerable difficulties in sample preparation since the analytes must be extracted and transferred to a liquid phase before introduction into the GC. Early techniques focused on simply washing the air-dried solid matrix with organic solvent but these methods proved to yield low extraction efficiencies (analyte recoveries were considerably less than 100 percent). The gold standard for the extraction of analytes from soil and sediment matrices is the Soxhlet extraction technique. The Soxhlet is a glass distillation setup that repeatedly passes pure solvent through the soil/sediment matrix over a period of 24 to 48 hours. After this time, the solvent is collected and the volume is reduced and analyzed by GC.

Laboratory studies have recovered approximately 100 percent of analytes with this method but Soxhlet glassware is expensive (each setup costs at least $300), it uses expensive organic solvents (approximately $150 per four liters), and is very labor and time intensive. Alternatives to the Soxhlet technique include relatively rapid sonication procedures and automated heated solvent extraction systems.

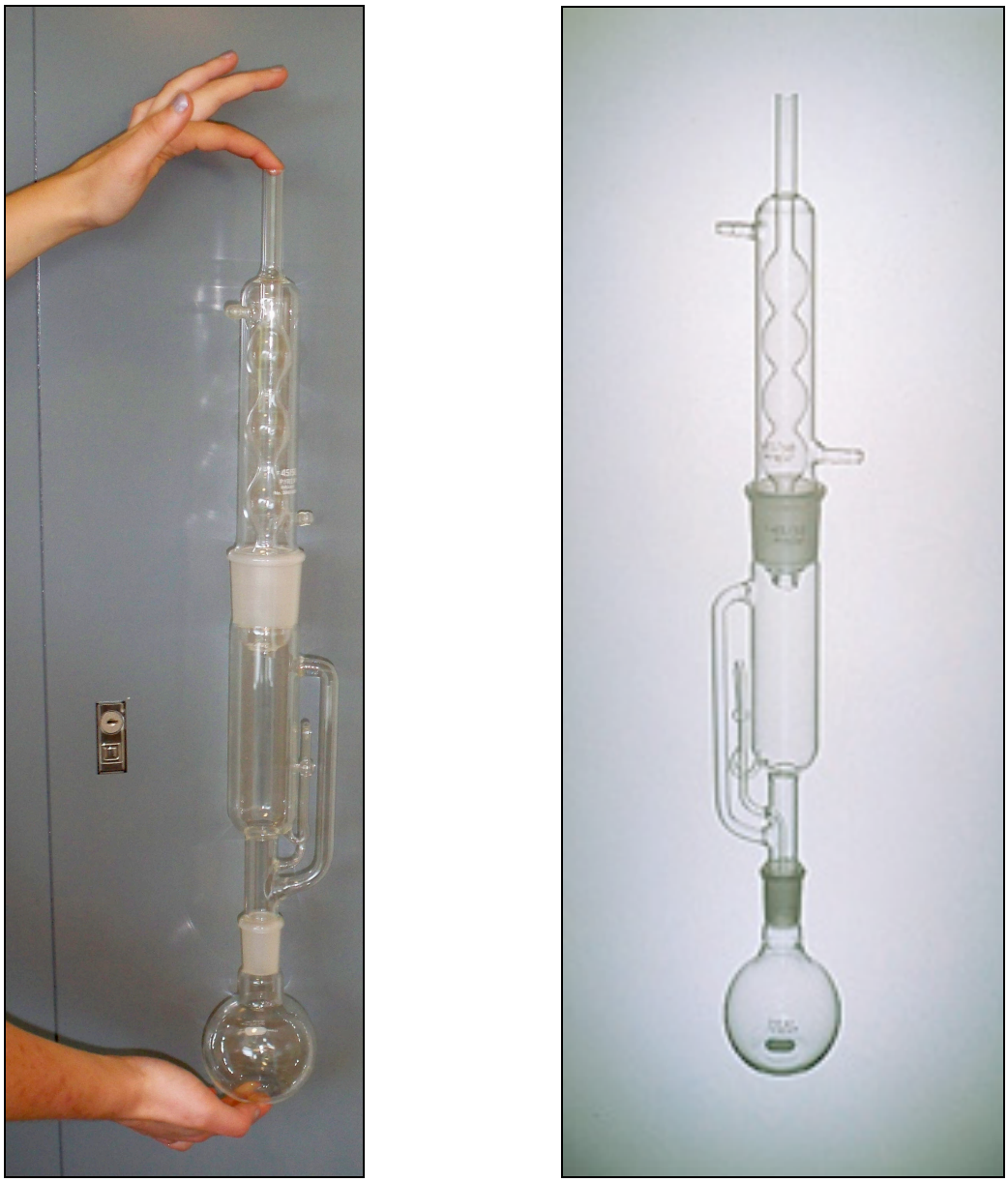


Figure 2.4 Soxhlet Extraction Glassware. Reprinted with permission from VWR Scientific Products International, Chemglass Life Sciences, and Corning.

2.2.4 Biological Samples

Biological tissue samples are undoubtedly the most difficult to extract and analyze. During the extraction process, the analytes need to be effectively transferred from the outside and inside of cellular matter to the solvent phase. The approaches used are as diverse as the high number of sample tissue types. Common approaches include (1) drying the tissue, followed by grinding, and Soxhlet extraction and (2) a combination of grinding and sonication, followed by liquid extraction. Whichever method is used, extensive sample cleanup (the removal of interfering substances and analytes) is necessary since the analyst should not inject non-volatile biological material into a GC.

An additional point should be made here. Gas chromatography is only used for analytes with boiling points below approximately 300 °C and this limits the utility of GC analysis for both the organic and analytical chemist (HPLC was developed for most other non-volatile compounds). However, some analytes can be reacted with derivatizing agents to remove functional groups that tend to make them nonvolatile. A common derivatizing agent (also referred to as a silylating agent) is N,O-bis (trimethylsily) acetamide which converts groups such as -OH, -COOH, -NH_2, =NH, and -SH to a -O-Si$(CH_3)_3$ group that renders the compound volatile. It should be noted that derivatizing agents are very hazardous and usually carcinogenic.

2.2.5 Analyte Recovery

Now that we have presented some of the common extraction techniques, another problem must be pointed out. How is it possible to know all of the analytes were extracted from the sample (i.e. water, urine, soil, fish)? This question becomes more difficult to answer as the sample matrix becomes more complex. For example, how does the chemist quantitatively recover all of the analyte from lake sediment or from food items? These sample matrixes can have analytes contained within every clay particle or biological cell and require the development and testing of rigorous extraction procedures. Fortunately, many of these procedures have been developed and are published by governmental agencies, industry, or research scientists. As a result, incorporation of these procedures into the laboratory is relatively easy. As an aid to determining how well your extraction procedure works, relatively expensive “reference” samples that contain a known amount of analytes can be obtained for a variety of sample

matrixes (i.e. fish, sediment, and manufactured goods). A procedure can be validated if the results from your method are statistically equivalent to the known concentration. For many procedures, it is not necessary to have a high recovery (i.e. 98%) but it is necessary to have a known and consistent recovery, even if it is low.

In addition to the potential human errors present in an analysis, instrument detectors can also contain errors due to non-optimum instrumental setting, out-of-date tuning or calibration, and when peaks elute from the column with more than one analyte or in mass spectrometry when more than one reference spectra is identified in the computer search library. This latter situation is common with low concentrations of analytes.

Now that the basic problems and common errors associated with sampling handling and instrumentation have been identified, we will move on to distinctions between gas and liquid chromatography. Gas and liquid chromatography were originally developed due to the existence of two basic different types of analytes: (1) those that are thermally stable (do not degrade at temperatures up to 300 °C) and are volatile at relatively low temperatures (below 300 °C), and (2) for analytes that are not volatile and/or thermally degrade at temperature above room temperature. GC is used for thermally stable and volatile chemicals while HPLC is used for both non-volatile compounds and ones that degrade at high temperatures. Recent advances in the stationary phases on separation columns and mobile phase selection (solvent gradient in HPLC) allow many analytes that were exclusively analyzed by GC to be analyzed by HPLC. For example, GC was the exclusive technique for analyzing mixtures of volatile organic solvents. Yet today, by changing HPLC to a reverse phased system (where the separation column is the nonpolar phase and the solvent is the more polar phase) it can now analyze components of organic solvents. HPLC will be discussed in depth in the next chapter.

GC analysis can also have special concerns. Impurities introduced during sample preparation can result in contamination that may interfere with the analysis of a desired analyte or introduce additional peaks into the chromatogram (the output of a chromatograph). A notable case is a class of compounds known as phthalates that are found in plastics that interfere with the analysis of chlorinated pesticides such as DDT and PCBs in GC analysis with an electron capture detector (ECD). Even with detection by mass spectrometry, the analysis may conclude that these compounds were present

in the original samples when in fact it they are laboratory contaminants. As a result, contact with plastics must be avoided regardless of the detector that is used. It is also important to purchase GC grade solvents (at over $150 per four liters) that are certified to contain an extremely low amount of impurities when trace analyses are being conducted.

Some functional groups of analytes, such as in the analysis of Bisphenol A, a known endocrine disruptor present in some plastic bottles, may react with or irreversibly adsorb to the glass surfaces in the GC injector liner and result in the analyst reporting the absence of Bisphenol A in a sample when in fact it was present but lost during the analysis. This can be overcome by deactivating the surfaces with a silanization agent that coats the glass with a non-reactive trimethylsilane group, and allows the analyte(s) to pass through the system to the detector. What and when to worry about these problems, and many others, come with experience and knowledge of the literature.

2.3 The Gas Chromatograph

The main purpose of chromatography is to separate a complex mixture of compounds into discrete chromatographic peaks containing only one analyte. Today's capillary column chromatographic systems are ideal for this task and interface well with detection by mass spectrometry due to the low volume of carrier gas used in capillary columns (1 to 5 mL/min as opposed to 60-100 mL/min in packed column GC used prior to the 1980s). Figure 2.5 below, illustrates the major components of a modern capillary column gas chromatograph – mass spectrometry (GC-MS) system.

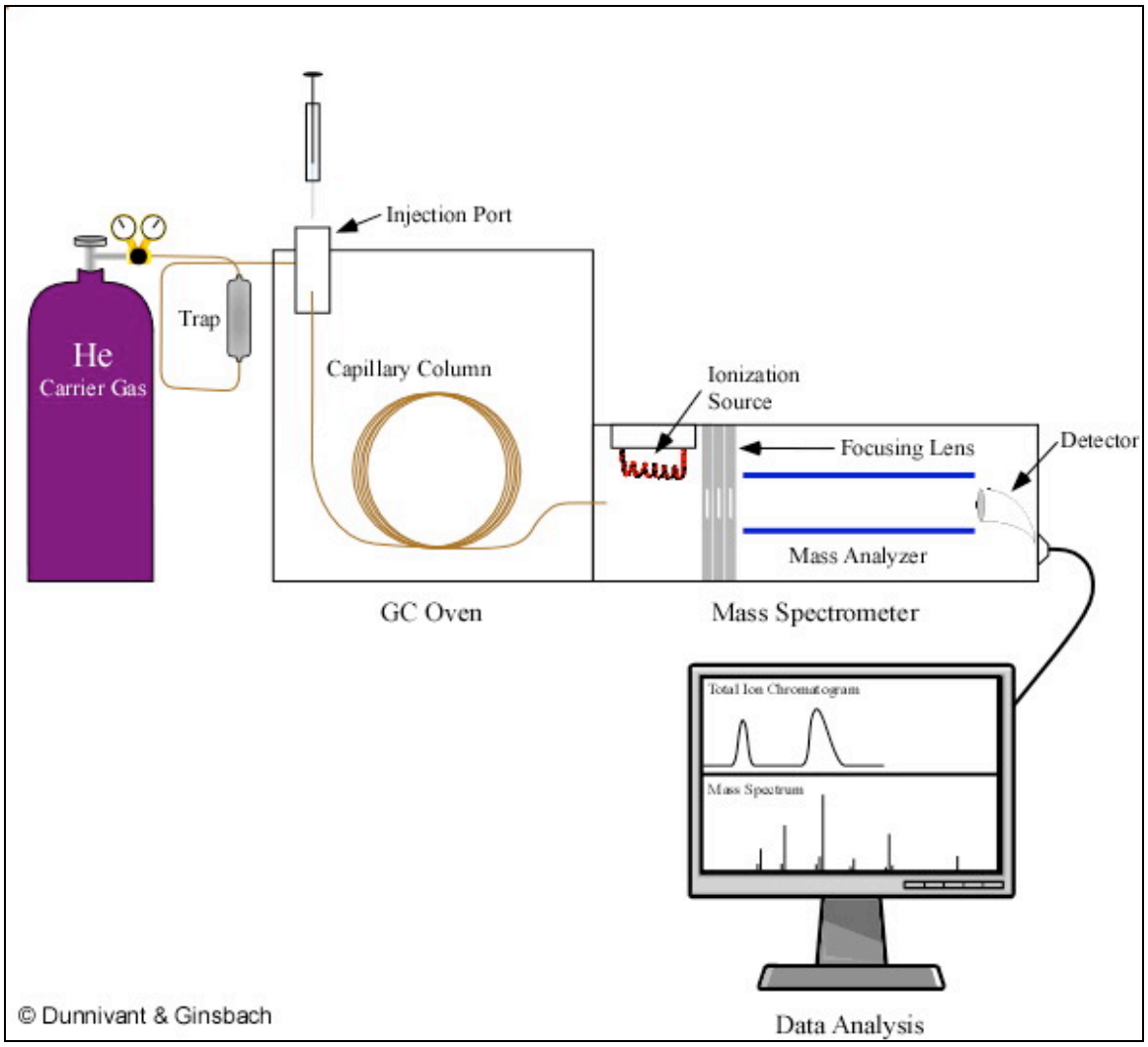
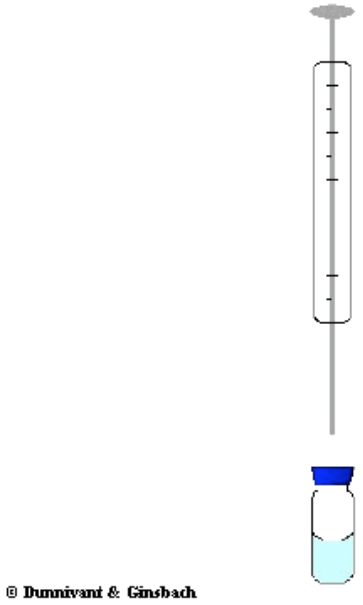


Figure 2.5. A GC-MS System

2.3.1 Carrier Gases: The first important component is the carrier gas or mobile phase. For a basic GC system, extremely pure helium is usually used, and hydrogen is less frequently encountered due to its explosive nature. Helium is used due to its inertness, non-reactive nature, and the shape of its van Deemter curve that allows for a relatively wide range of optimum mobile phase linear velocities. The common grade of helium used is referred to as "five-nine gas", meaning that it is 99.999% pure. But this level of purity is still not sufficient for most systems when trace (parts per million or parts per billion) analyses are being conducted. Before entering the GC, the 2500 psi (18 000 kPa) pressure in the gas cylinder is reduced to approximately 60 psi (400 kPa) with a two-stage regulator before entry into the GC. But first, the He gas is passed through at least one resin trap to further remove hydrocarbons, oxygen, trace analytes, and/or water vapor

that could interfere with analysis, degrade the column or interfere with the detector.

2.3.2 Injectors: After passing through the purification traps, helium enters the injector where it acts as the mobile phase and helps "push" the analytes through the analytical (separation) column. A variety of injectors are used in GC, but this text is concerned with the most common, a split-splitless injector. This type of injector can be used in two modes. For solutions containing extremely concentrated levels of analytes (in the parts per thousand or percent level as is encountered in synthesis operations), the injector is operated in the split mode. In this mode only a small fraction of the 0.2 to 1 μL of solution injected actually enters the separation column and the majority of the sample is vented to the atmosphere. The high concentration of analytes in the solvent allows for adequate identification and quantification. For solutions containing lower levels of analytes (parts per million and parts per billion), the injector is operated in a dual or splitless-split mode. Upon injection of a sample, the injector is operated in a splitless mode where all of the injected volume is being pushed onto the column. But if this mode of operation is allowed to continue throughout the chromatographic run, the peaks will be non-symmetrical (they will tail or be skewed) which will interfere with peak integration because of a continual addition of solvent molecules entering from the injection port. To avoid this problem, the split mode is switched on approximately 30-60 seconds after injection. This splitless-split mode allows the majority of the sample to "load" onto the column while clearing out the remainder of the sample to allow for a "clean", well-shaped chromatographic peak. A typical split-splitless is shown in Animation 2.1.



Animation 2.1 Illustration of the Spiltless-Split Mode of Injection.

A few more points need to be made about the injector inlet. The interface between the atmosphere and the injector is separated by a septum such as the ones shown in Figure 2.6. Septa are manufactured out of various materials, all of which must be inert to leaching organic constituents into the GC or are coated on the GC side of the septum with Teflon. Septa are inexpensive and are routinely replaced, usually after 30 to 50 injections.

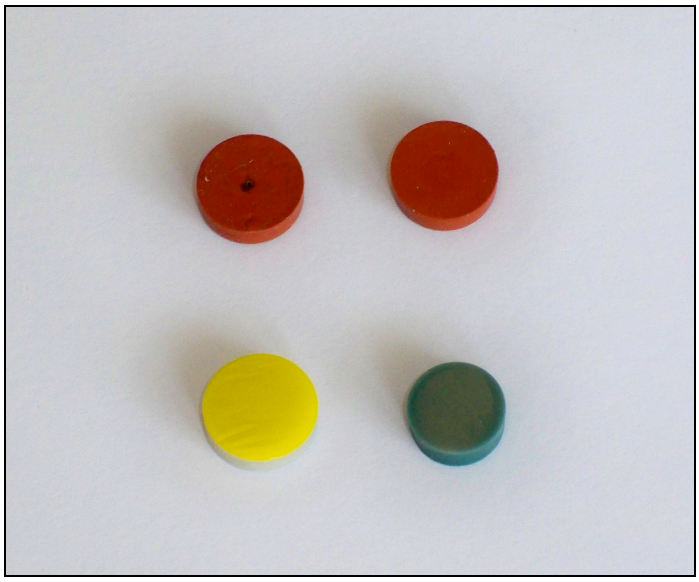


Figure 2.6 GC Septa. Note the puncture holes in the top left septum. The lower left septum contains a Teflon coating (yellow). The septa shown here are about 1 cm in diameter.

Samples are injected through the septa and enter a glass liner in the injection port. The purpose of the glass inserts (liners) is to avoid exposure of the analytes to reactive hot metal surfaces such as those contained in an unlined injector. Inserts come in a variety of forms. All liners contain a hole in the top to allow entry of the injection needle, a wider middle space for the expansion of liquid solvents into the vapor phase, and a hole in the bottom for insertion of the capillary column. Glass wool is usually present in the glass insert to keep pieces of the septum from blocking the inlet of the capillary column and to trap non-volatile components of the sample. Figure 2.7 shows two common injector inserts, one with the glass wool in the middle and one with the glass wool at the end.

Figure 2.7 Injection Glass Inserts. The insert on the right shows the o-ring that seals the injection chamber and forces carrier gas through the column.

As noted earlier, samples are typically introduced into the GC with a glass syringe with a metal needle. Samples can be injected manually or with an automatic sampler. Standard 10-µL syringes are shown in Figure 2.8.

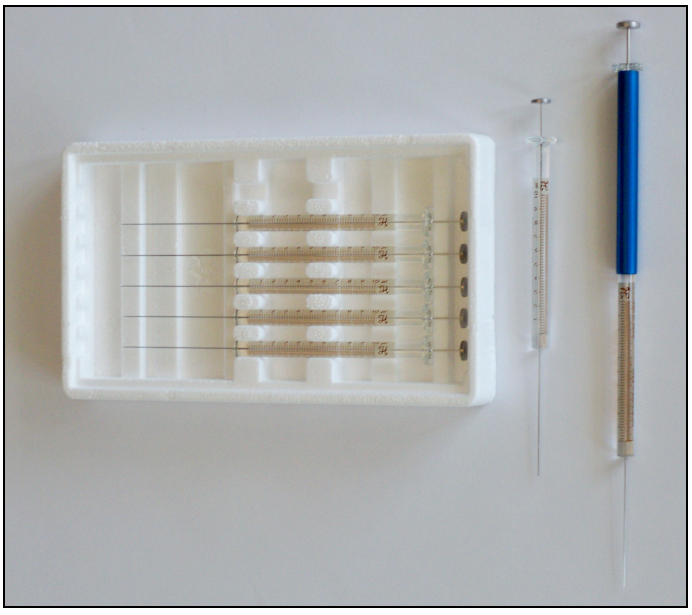


Figure 2.8 GC Injection Syringes.

All connections in GCs, from the carrier gas cylinder to the detector are made with Swedge Lock (a.k.a. Swagelok) fittings that seal the connections at high gas pressure. These fittings consist of a threaded nut, back ferrule, and front ferrule, all placed around a piece of tubing (refer to Figure 2.9). Fittings come in Teflon, stainless steel, and copper and in a variety of sizes ranging from smaller sizes for capillary columns as small as 0.2 mm to 6.0 mm packed columns. Ferrules are also available in graphite and in a variety of advanced materials such as Vespel, a composite of graphite and ceramic. A gas-tight fitting is achieved by tightening the nut and compressing the ferrule around the metal tube. A selection of ferrules and how they fit around a piece of tubing is shown in Figure 2.9.

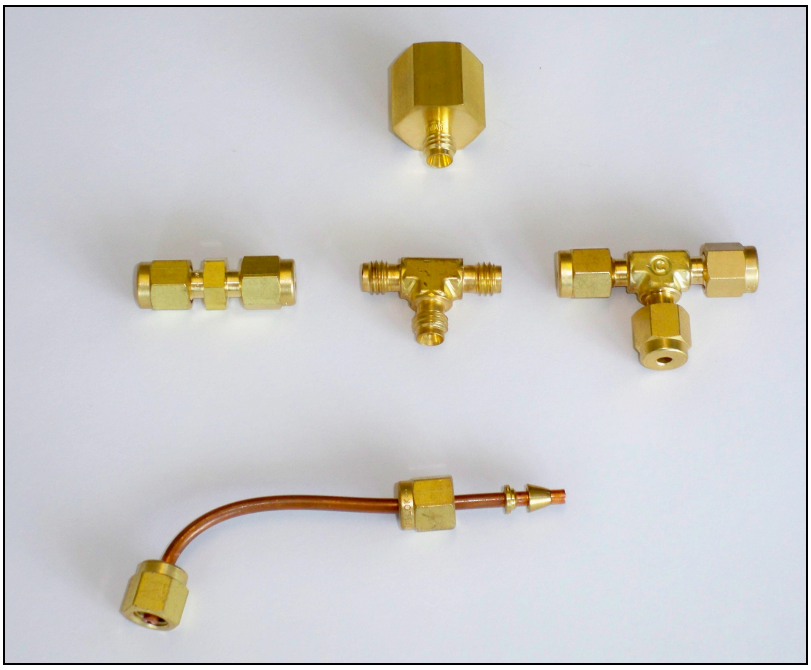


Figure 2.9 Swedge Lock Fittings.

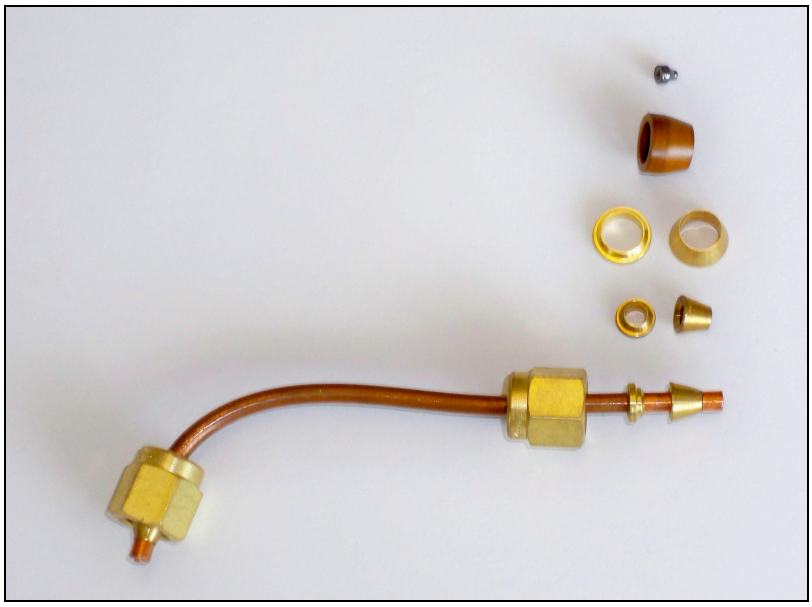


Figure 2.10 Several Types of Ferrules.

In addition to the split-splitless injector, other types of injector systems are available including an on-column injector and a cryogenic focusing injector. In the past, when packed columns were used, most injections were made directly onto a section of the column that did not contain any stationary phase resin. This concept has been extended to capillary column technology by using a wide-bore column (typically 0.5 mm in diameter or greater). A syringe with a very narrow capillary column needle (0.2 mm in diameter) is placed through a special port at the head of the injector and liquid samples are placed directly onto the column. The needle is withdrawn, the injector port is sealed and the chromatographic run is started. On-column injection avoids exposing the analytes to any reactive surfaces.

Highly volatile analytes, normally not separated by standard GC conditions can be analyzed using a cryogenic focusing injector. Here, gas or liquid samples are injected through a septum, but the bottom of the injector contains a cryogenic fluid (liquid N_2) around the head of the capillary column. The cryogenic fluid cools the column and causes the analytes to condense at the head of the column. After injection, the cryogenic fluid is removed, the column oven slowly heated, and the volatile analytes are analyzed.

With today's extremely small injection volumes (0.2 to 1 μL), reproducibility of sample injection can be a problem. It is necessary to inject exactly the same volume of sample (to within three significant figures) to avoid introducing considerable error into the results. Two solutions have been devised for this problem: (1) automatic samplers/injectors and (2) internal standards. Mechanical automatic samplers can accurately and consistently reproduce small volume injections and save considerable labor costs (and time if you happen to be a graduate student or on a slim budget). A typical automatic sampler today can hold up to 100 samples and be programmed to run the samples in any order. This is more convenient than manually injecting a sample and waiting for the GC run to end, which can range anywhere from 5 minutes to hours. The automatic sampler allows the user to simply return hours to days later to find the samples analyzed and the data stored and ready for processing on the computer-controlled station. Automatic samplers are common features today even in graduate schools since they run hour after hour, day after day, with minimal oversight or maintenance. The typical cost of an automatic sampler is only $10,000 and they rapidly pay for their self in high sample volume work environments.

The second option for overcoming injection errors, an internal standard, is also common in capillary GC and is usually used in conjunction with automatic samplers. Internal standards were discussed in section 2.1 but will be repeated here due to its importance. An internal standard is a chemical (analyte) that is not originally present in any sample. Equal amounts of the internal standard are added to every sample and reference standard. The computer program in the control station can then be programmed to correct for injection errors. One way to use this technique is to average the peak area for the internal standard that is measured in every external standard and compare the area to that observed for each sample. If the internal standard measured in a sample falls below or above this average due to a poor injection, the computer will automatically adjust the areas of every peak in the chromatogram accordingly. By using a combination of automatic injectors and internal standards, highly accurate and consistent results can be obtained.

2.3.3 Columns/Ovens: Separation columns are the heart of the GC and are housed in a temperature controlled and temperature programmable oven that can control temperatures to within 0.5 C. Considerable advances were made in gas chromatography in the 1980s, especially with regard to columns. Prior to the advent of capillary columns, chromatographic systems

used packed columns. Packed columns are 1/8 to 1/4 inch metal, Teflon, or glass tubes filled with an inert resin coated with the stationary phase. Early stationary phases were highly viscous, non-volatile liquids that interact with the analytes to achieve separation or were molecular sieves that separated the analytes by molecular size and diffusion. In the early 1980s, packed columns were mostly replaced with capillary columns that are open tubular columns (internal diameters in the tenths of millimeters) with the stationary phase placed on the column walls. Initially, stationary phases were simply applied to the walls as non-volatile liquids, however today most phases are covalently bonded to the fused silica wall which yields more thermal stability. Capillary columns have dramatically more theoretical plates than packed columns and greatly improve resolution. The reader should recall this relationship from Example 1.1 in section 1.2. For example, capillary columns can have as many as 1000 times more theoretical plates as compared to a packed column. Figure 2.11 shows several GC columns.

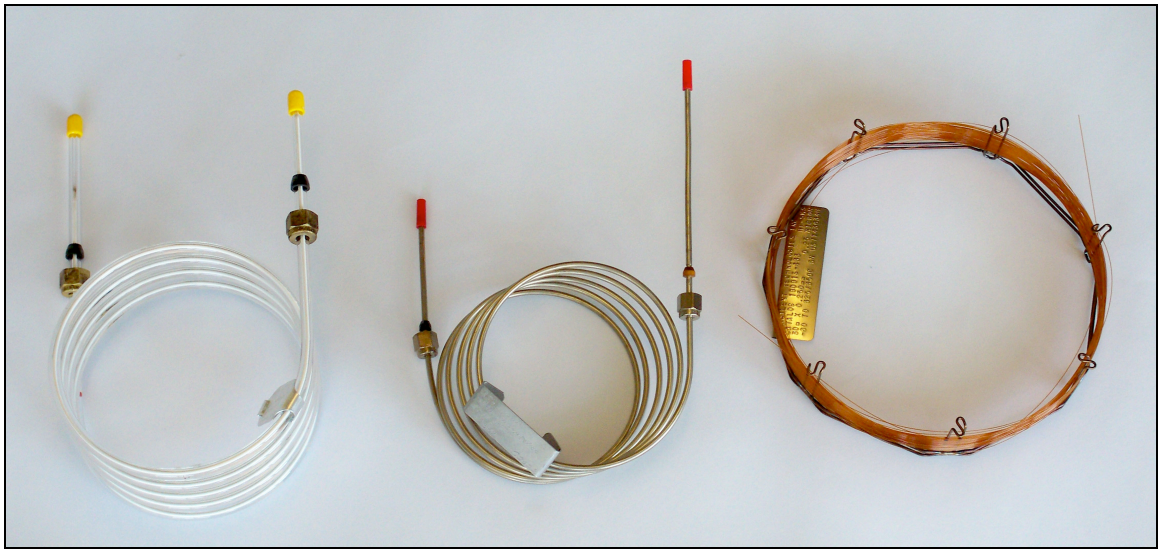


Figure 2.11 A 1/4-inch Glass Packed GC Column, a 1/8-inch Stainless Steel GC, and a Fused Silica Capillary Column (from left to right).

Figure 2.12 Resin for GC Packed Column.

A summary of the most common stationary phases is given in Table 1.1. The selection of a particular phase depends on the intermolecular interactions expected for the analyte of interest. As discussed in Chapter 1, the selection of the phase follows the adage "like dissolves like" or in this case "like stationary phases attach to like chemicals." The uses of each resin are also shown in Table 1.1. Additional stationary phases are available for a variety of analyte applications. Even some chiral compounds can be separated with specialty stationary phases.

Table 1.1. Common Stationary Phases and Their Primary Use

STATIONARY PHASE	APPLICATIONS
Polydimethyl siloxane (Trade names are DB-1, HP-1, OV-1, and SE-30)	This is a general purpose nonpolar phase for separating hydrocarbons, polynuclear aromatics, nonpolar drugs, chlorinated pesticides, and PCBs
Poly(phenylmethyldimethyl) siloxane (5-10% phenyl) (Trade names are DB-5, HP-5)	Still mostly nonpolar but with some polarity. Used to separate fatty acid methyl esters, alkaloids, drugs, and halogenated chemicals
Poly(phenylmethyl) siloxane (50% phenyl) (Trade names are DB-15 and OV-17)	Slightly more polar. Used to separate more polar drugs, pesticides, and glycols

Poly(tritluoropropyldimethyl) siloxane (Trade names are DB-210 and OV-210	More polar. Used to separate chlorinated aromatics nitroaromatics, and alkyl-substituted benzenes
Polyethylene glycol (Trade names are DB-WAX and Carbowax)	The glycol functional group makes this phase considerably polar. Used to separate free acid, alcohols, ethers, essential oils, and glycols
Poly(dicyanoallyldimethyl) siloxane (Trade names are DB-1701 and OV-275)	The most polar phase shown here. Used to separate polyunsaturated fatty acids, free acids, and alcohols

Today most columns are fused silica capillary columns with typical internal diameters ranging from 0.25 to 0.53 mm. Column lengths range from 5 to 100 meters. As noted in Chapter 1, the longer the column, the more theoretical plates it will contain and therefore long columns are capable of separating almost any mixture of compounds. Film thicknesses range from 0.25 to 3.00 μm with thicker films usually providing more resolution (and longer analysis times). Cross-linking of films is also common and provides more thermal stability and less "column bleed" of the stationary phase. Lower column bleed provides for a more stable detector baseline and these columns are preferred in mass spectrometer applications.

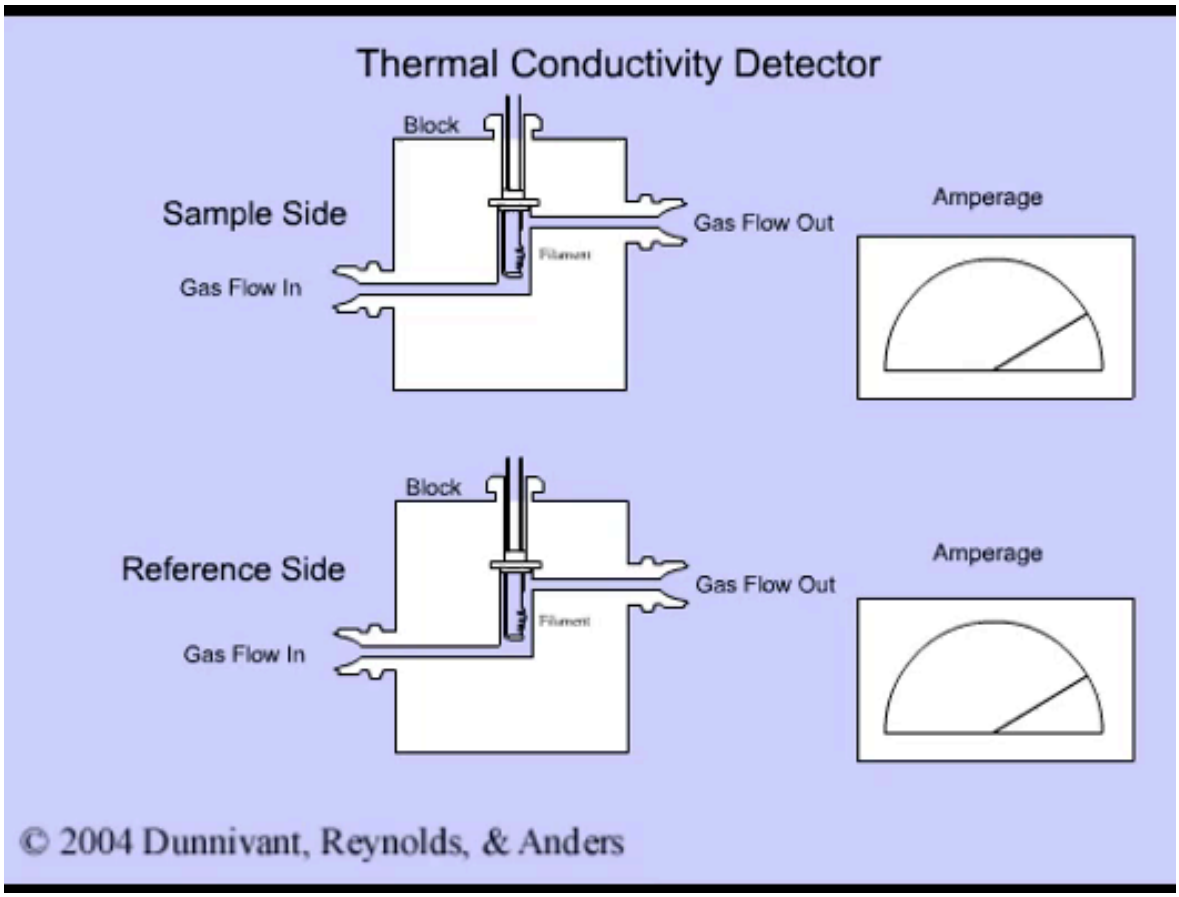
2.3.4 Detectors: While the focus of this book is utilizing mass spectrometry (Chapter 4) as the detector, it is informative to note that a variety of detection systems are available for GC. The most common and commercially available ones are listed in the table below with information on their detection limits and analytes of interest.

Table 2.2 Commercially Available GC Detectors.

Detector	**General Type**	**Analytes it is used to measure**	**Typical Detection Limits**
Flame Ionization Detector (View the FID Animation below)	Selective	Any chemical that will burn in a H_2/O_2 flame	parts per million
Thermoconductivity	Universal	Any chemical	parts per

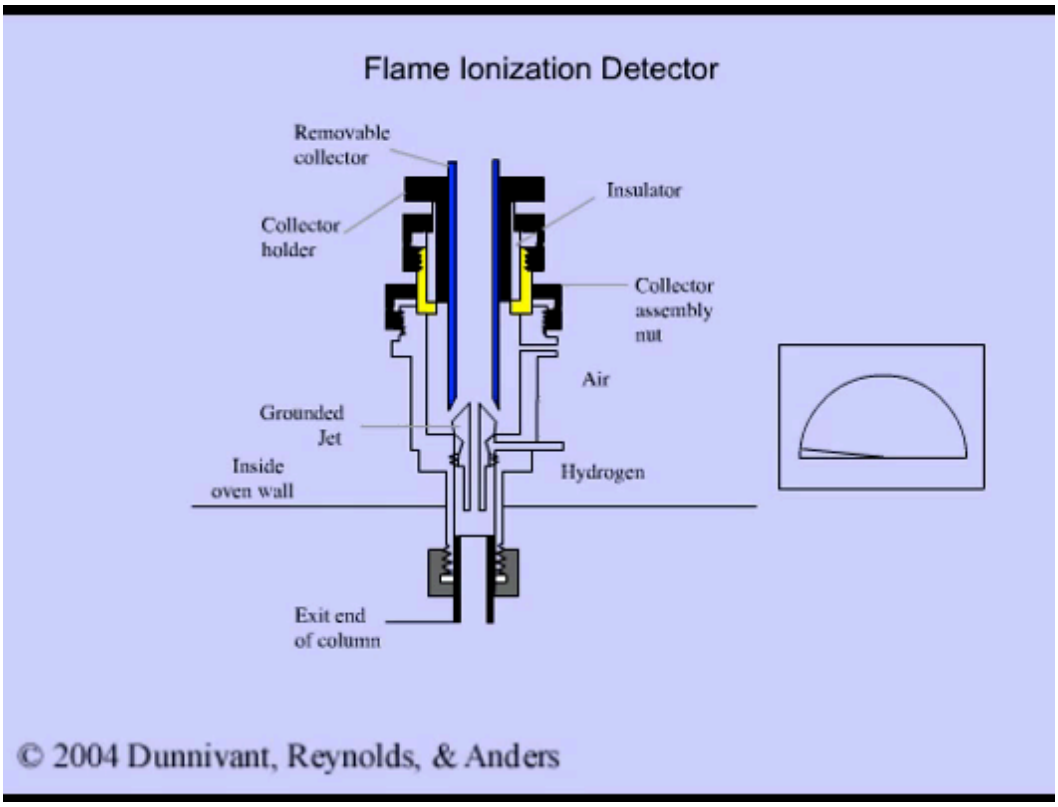
Detector (View the Thermocon-ductivity Animation below)		with a thermal conductivity (~specific heat) different from He	thousand or hundred
Electron Capture (View the ECD Animation below)	Selective	Electrophores such as halogenated hydrocarbons	parts per billion or less
Flame Photometric	Specific	P and S containing compounds	parts per million or less
Fourier Transform Infra-Red	Specific	Chemicals with specific molecular vibrations	parts per thousand or hundred
Mass Spectrometry	Universal	Any chemical species	parts per million or less

Three of the most common GC detectors will be discussed in detail here, while types of mass spectrometers will be presented in Chapter 4. One of the earliest GC detectors was the thermal conductivity detector (TCD). The basis for this detector is that most analytes have a thermal conductivity lower then that of helium. Helium is used as the carrier gas and as it passes a wire with a current applied to it the wire heats up via electrical resistance. Helium molecules remove the maximum amount of heat and the wire reaches thermal equilibrium and a constant current reading. As an analyte with a different thermal conductivity enters the detector, the wire heats up with increasing electrical resistance and the measured current decreases. Since the analytes pass through the detector as a "chromatographic plug" a bell-shaped current reading results known as a chromatographic peak. After the analyte has passed through the detector, the current returns to the original baseline reading as the helium re-cools the wire. Usually two matched columns and detectors are used, where only He is passed through one setup and samples are injected into the other. While this detector responds to any chemical with a thermal conductivity different than helium (which includes almost every other compound), these detectors suffer from relatively poor detection limits (parts per thousand to parts per hundred). Animation 2.2 illustrates the operation of a TCD.



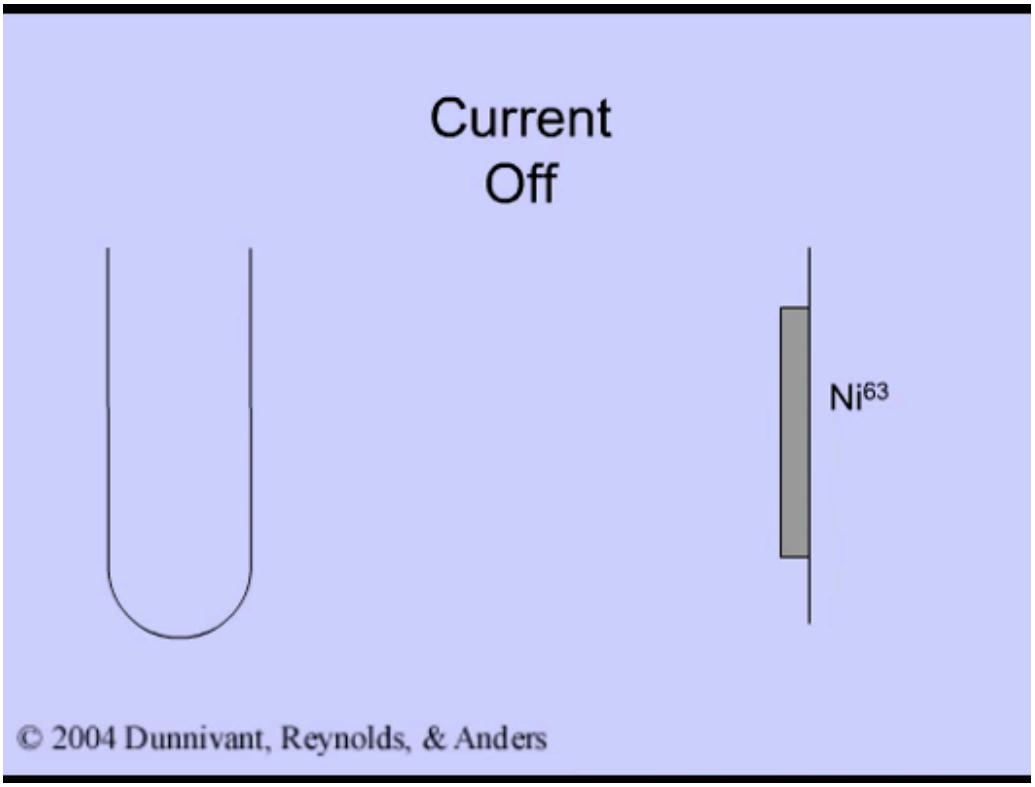
Animation 2.2 Illustration of a Thermal Conductivity Detector.

The most common detector in gas chromatography is the Flame Ionization Detector (FID). This detector is based on the fact that most chemicals will burn in an H_2-air flame and current can be passed through the path of ions produced in the flame. Helium is again used as the carrier gas and analytes are injected in the standard split-splitless injector. As individual packets of analytes are separated in the column and enter the detector they burn in the flame. As illustrated below in Animation 2.3, a potential is placed across the flame jet and an electron collector plate is placed above the flame. As ions are produced, electrons are passed through the ion cloud, and a current is measured that is proportional to the mass of analytes produced in the flame. The FID is also considered a universal detector, although not every chemical will burn in an H_2-air flame. FID are relatively sensitive with detection limits of 1 ppm for most chemicals.



Animation 2.3 Illustration of a Flame Ionization Detector.

The electron capture detector (ECD) is perhaps the most sensitive detector for a GC and was developed primarily to detect chlorinated hydrocarbons in the environment. It relies on the electrophilic nature of halogens contained in an organic chemical, but can be used to detect other electrophilic-contained elements such as oxygen. The detector is a sealed unit and contains a radioactive isotope of nickel, ^{63}Ni. This isotope gives off a steady supply of beta particles that are essentially high speed electrons. These high-speed electrons collide with trace amounts of methane carrier gas that enters the column after the column effluent and produces slower speed electrons (thermal electrons). These thermal electrons are captured by the anode in the middle of the detector and provide a constant current in the absence of any electrophilic analytes. As electrophilic analytes enter the detector they attract the thermal electrons and carry them out of the detector. This removal process results in a lowering of the current measured by the detector and the change in current is measured as an inverse chromatographic peak. ECDs are extremely sensitive and yield detection limits of pg or sub-parts per billion concentrations in the injection solvent. The operation of an ECD is illustrated in Animation 2.4.



Animation 2.4 Illustration of an Electron Capture Detector.

2.4 Advanced GC Systems

Most commercial GC systems come with ports for at least two injectors, and therefore two columns and two detectors. This allows for versatility and some inventive operational designs. With a special ferrule, two columns with different stationary phases can be inserted into one injection port allowing two analyses per injection and confirmatory analysis.

Confirmatory analysis, with respect to chromatography, is usually restricted to mass spectrometry detection, but when a sample is analyzed on two columns with different stationary phases, the likelihood of two different compounds yielding the same retention time on both columns and the same response on identical detectors is highly unlikely. Thus, dual column analysis usually produces confirmatory identification.

More enhanced arrangements include detectors aligned in series where the effluent of one detector is passed into another detector. This arrangement does not necessarily provide confirmatory analysis, but does allow considerably more information to be collected about the analyte. Note that the first detector cannot be a destructive detector since the chemical integrity of the analyte must be intact for the operation of the second detector.

2.5 Applications

An almost endless variety of chromatographic separations are achievable today due to the diversity of analytical columns. Major column manufacturers and distributors provide very useful Internet sites that contain chromatograms for common analytes that can be used to help select the appropriate column for your needs. In addition, technical help is available from professional chromatographers at these companies for more complex separations. The chromatograms below were selected from the hundreds available from Agilent technologies at http://www.chem.agilent.com/en-us/Search/Library/Pages/ChromatogramSearch.aspx. There many excellent additional examples from chemical and chromatography suppliers.

One of the most common published lists of GC applications is for the analysis of environmental pollutants. The chromatographs below are for a variety of chemicals, analytical columns, and detectors. As you review these note the correlation between the intermolecular forces available to the analytes and the stationary phases used to separate them. Also note the GC detectors used for each type of analyte.

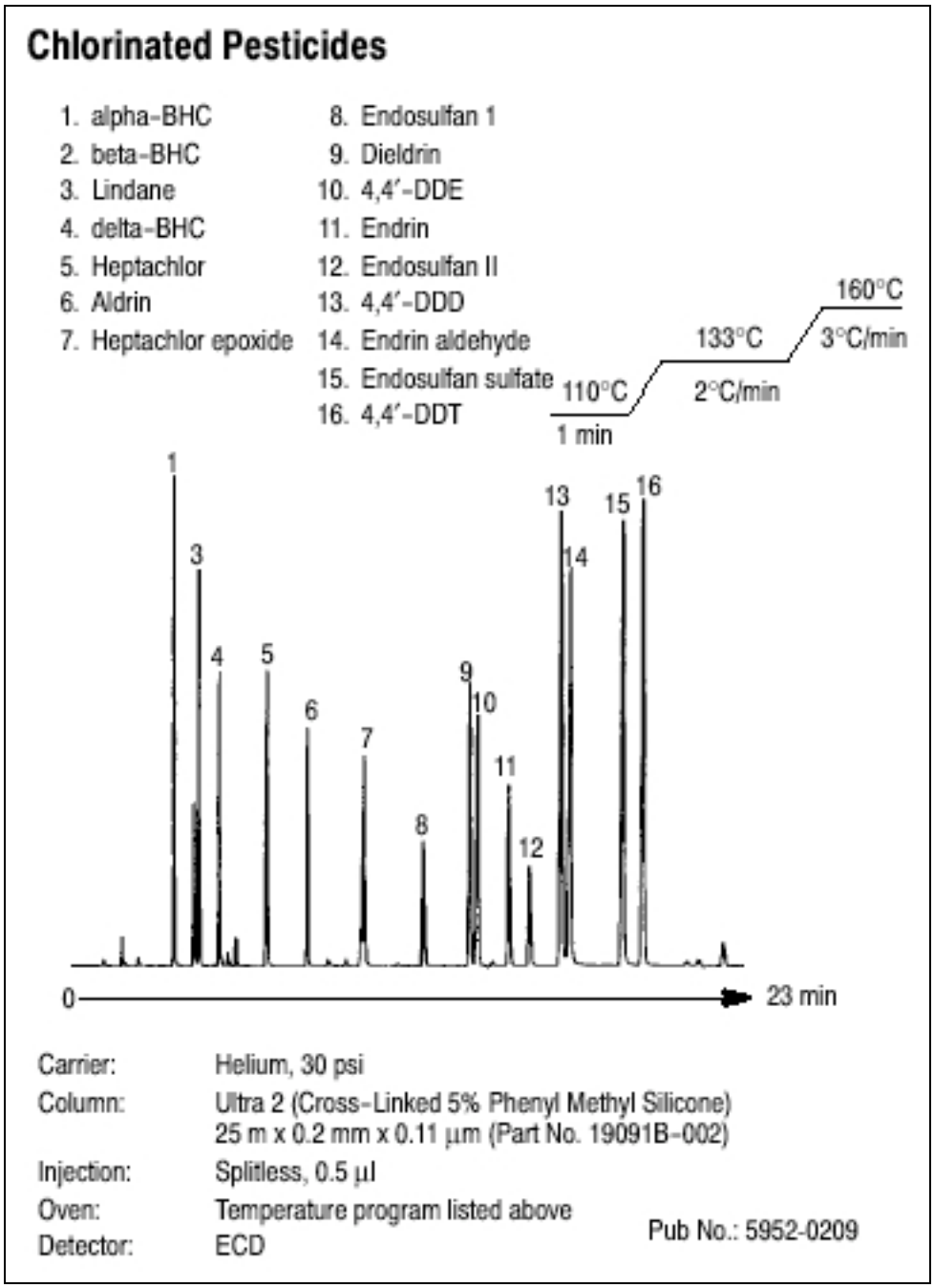


Figure 2.13 Analysis of Chlorinated Pesticides by GC-ECD using a Ultra 2 Column (5 percent cross-linked phenyl methyl silicone). Source: Copyright 2006 Agilent Technologies, Inc. Reproduced with Permission.

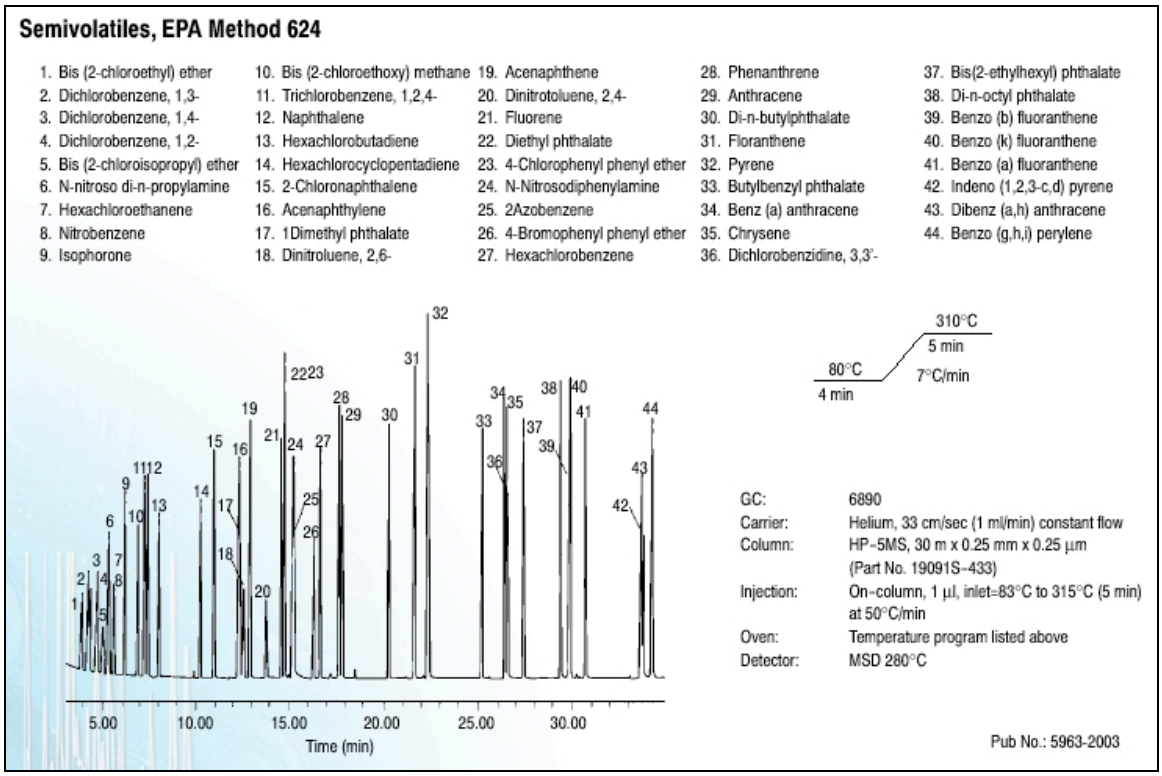


Figure 2.14 Analysis of Semivolatiles by GC-MS using HP-5 column. Note the large number compounds that can be separated in one GC analysis. Source: Copyright 2006 Agilent Technologies, Inc. Reproduced with Permission.

The pharmaceutical industry also heavily uses GC and HPLC to determine the purity of reagents, the identity of synthesis products, and the identity of medicines and illicit drugs. A few examples are shown below.

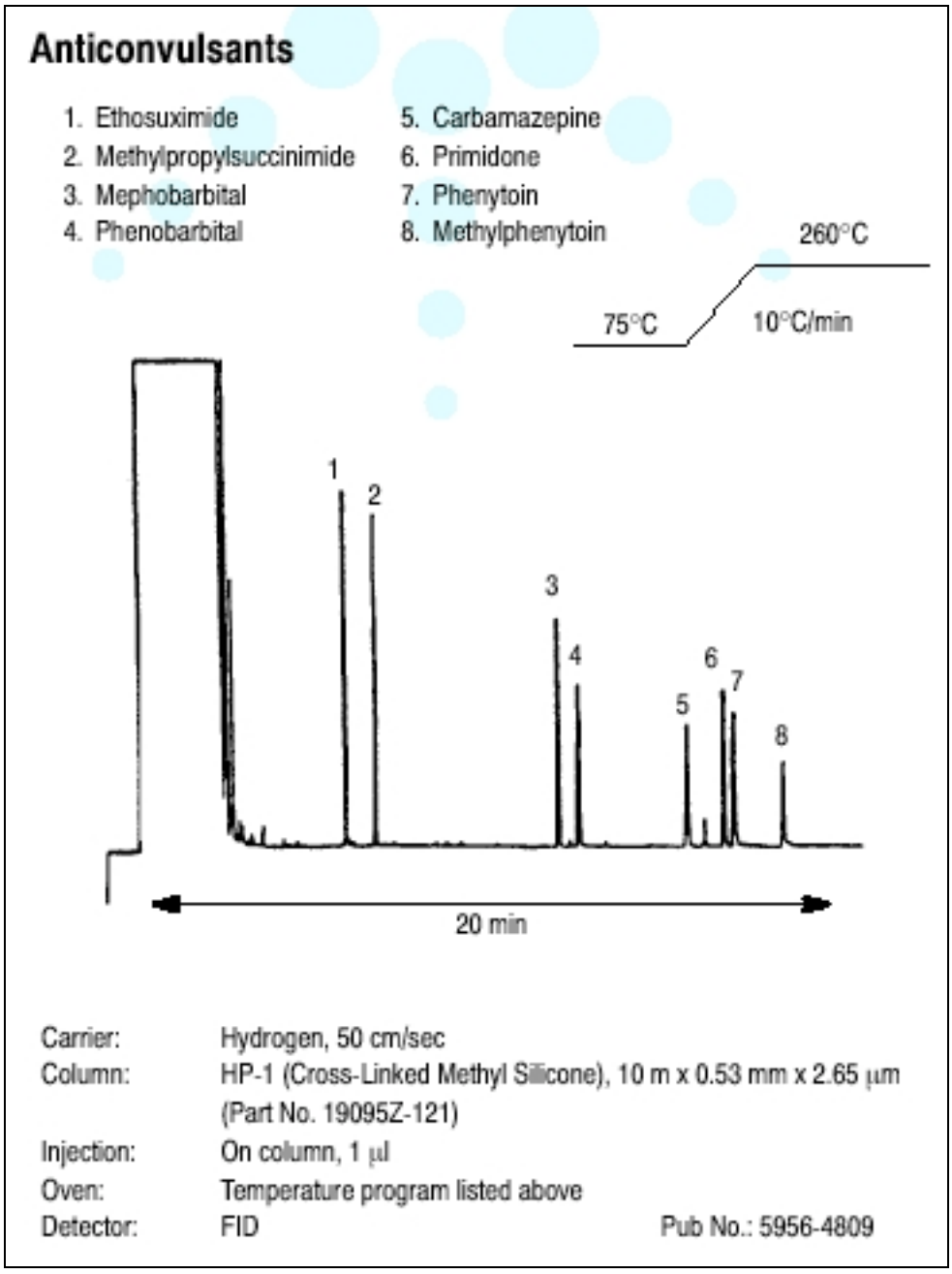


Figure 2.15 Analysis of Anticonvulsants by GC-FID using an HP-1 column. Source: Copyright 2006 Agilent Technologies, Inc. Reproduced with Permission.

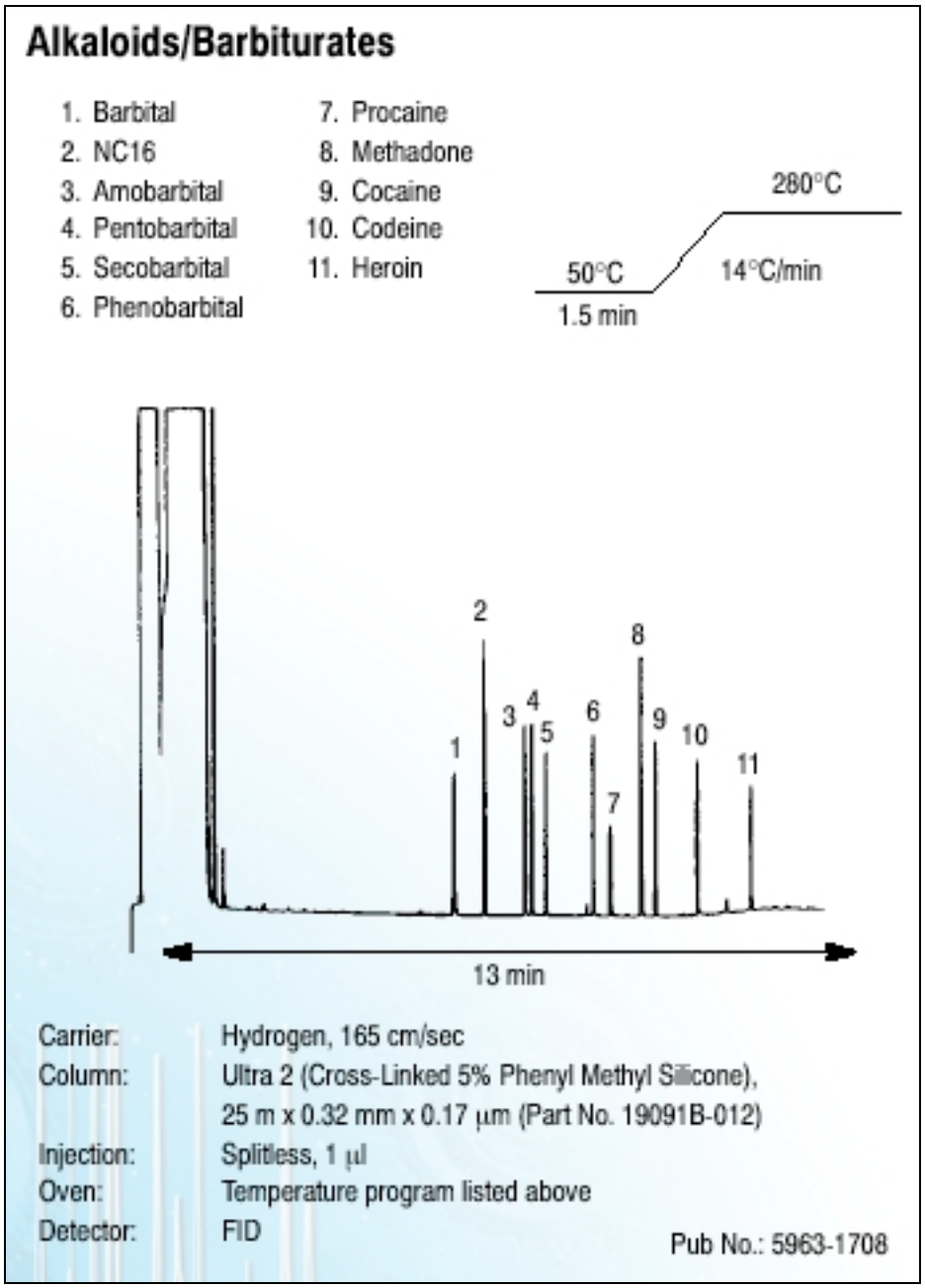


Figure 2.16 Analysis of Alkaloids and Barbiturates by GC-FID with an Ultra 2 column. Source: Copyright 2006 Agilent Technologies, Inc. Reproduced with Permission.

GC can also be used to determine the identity of natural products containing complex mixtures of similar compounds. For example, the geographic source of crude oil or natural gas can be determined by the "fingerprint", or relative distribution of major and trace compounds in each oil. Natural produce oils, such as food products or fragrances, can be

identified by GC-FID or GC-MS. A few examples of the separation of these complex mixtures are shown below.

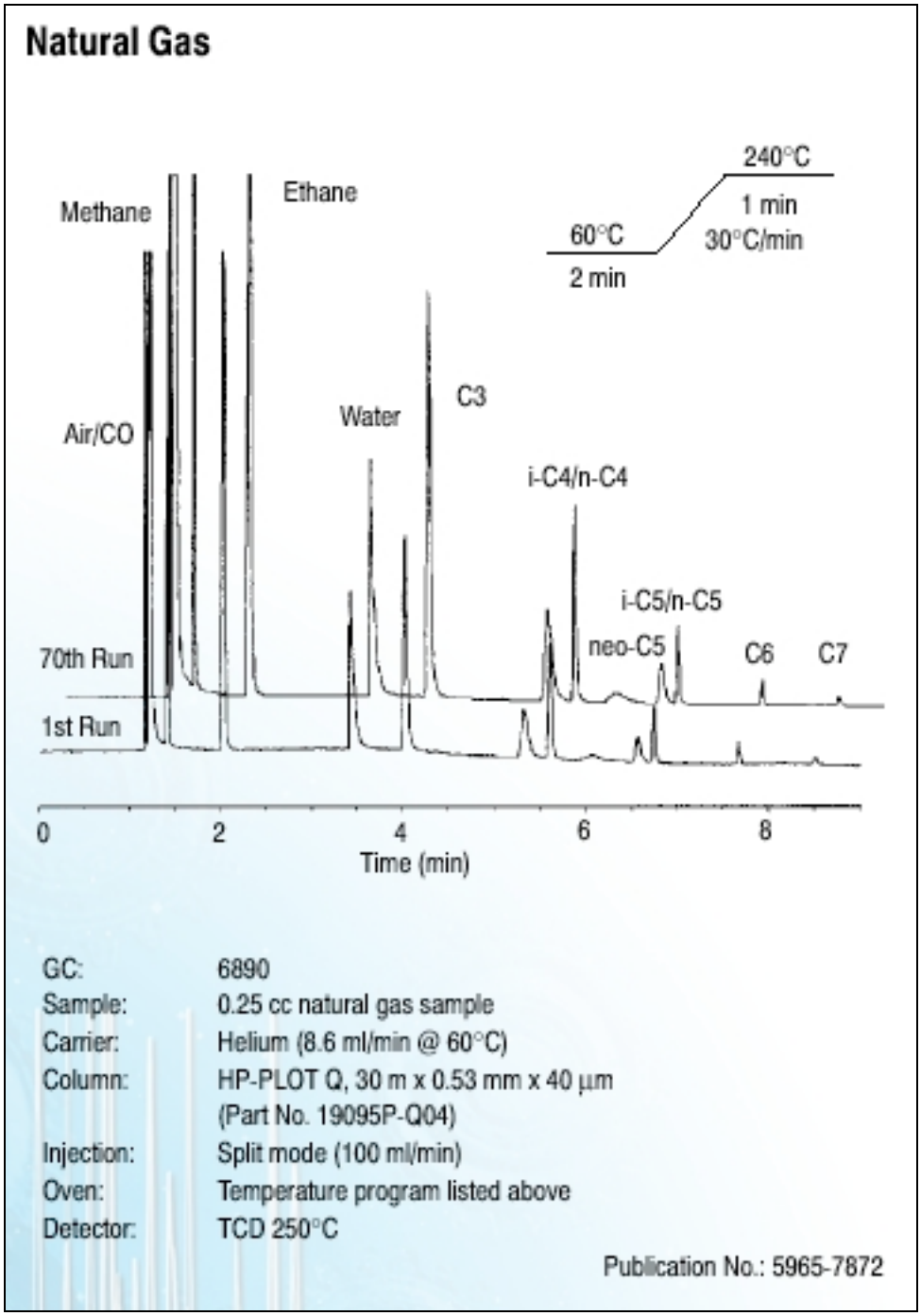


Figure 2.17 Analysis of Natural Gas by GC-TCD using a HP-PLOT Q column. Source: Copyright 2006 Agilent Technologies, Inc. Reproduced with Permission.

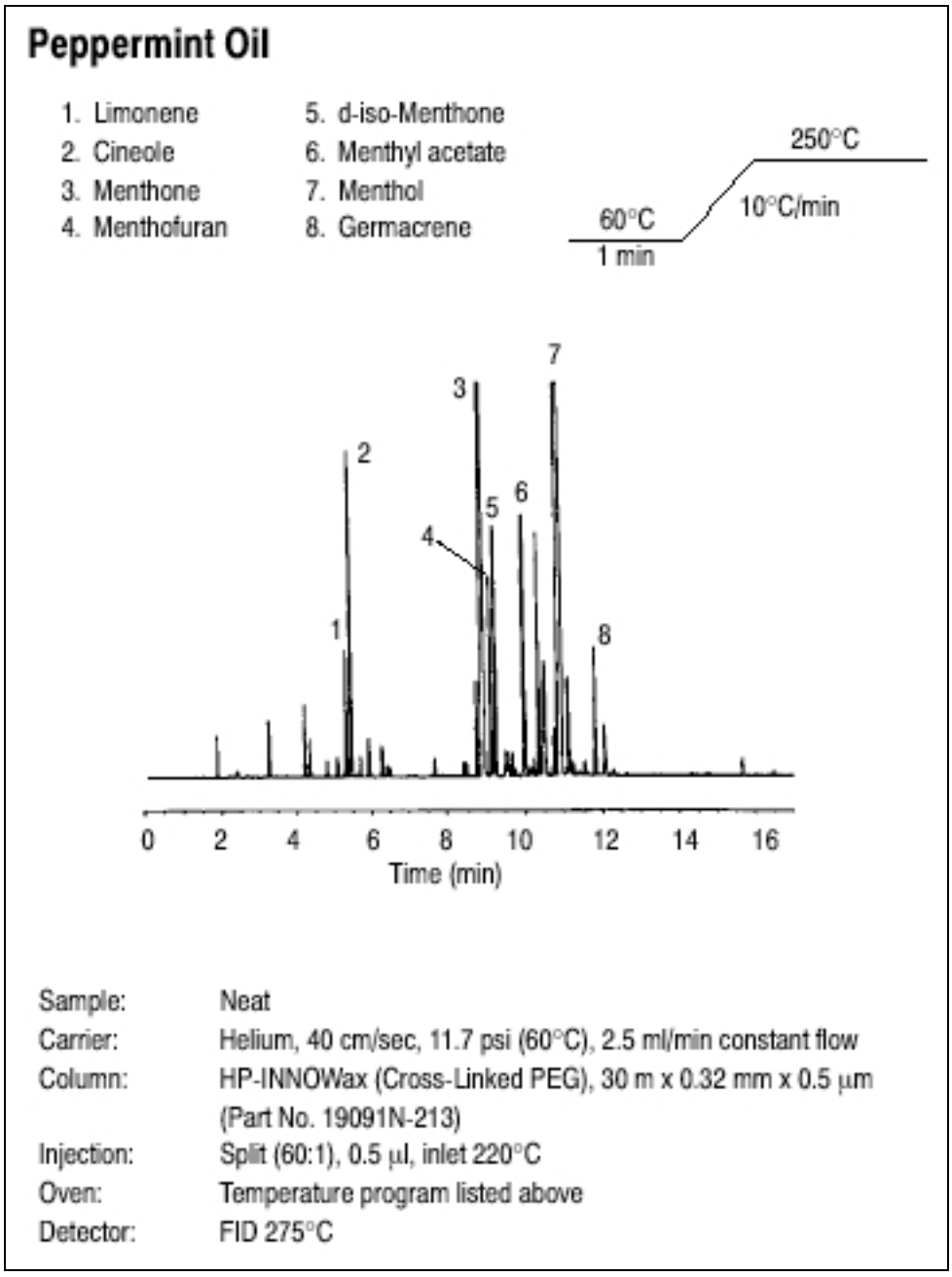


Figure 2.18 Analysis of Peppermint Oil by GC-FID using an HP-INNOWax column. Source: Copyright 2006 Agilent Technologies, Inc. Reproduced with Permission.

The purity of solutions, from relatively pure solvent such as xylene, to liquors such as scotch can be also be determined by GC. Two examples are shown below.

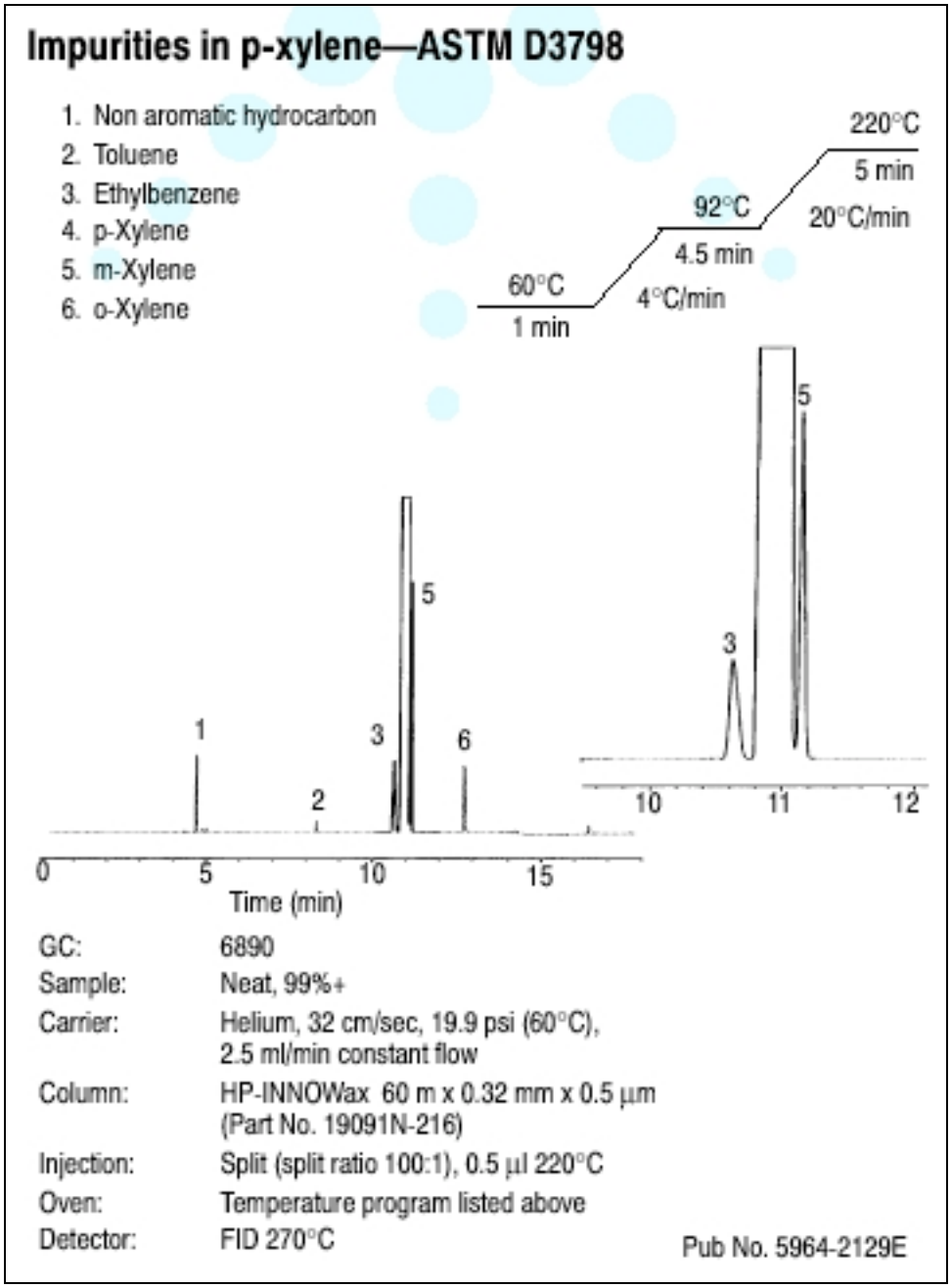


Figure 2.19 Analysis of p-Xylene by GC-FID using a HP-INNOWax column. Source: Copyright 2006 Agilent Technologies, Inc. Reproduced with Permission.

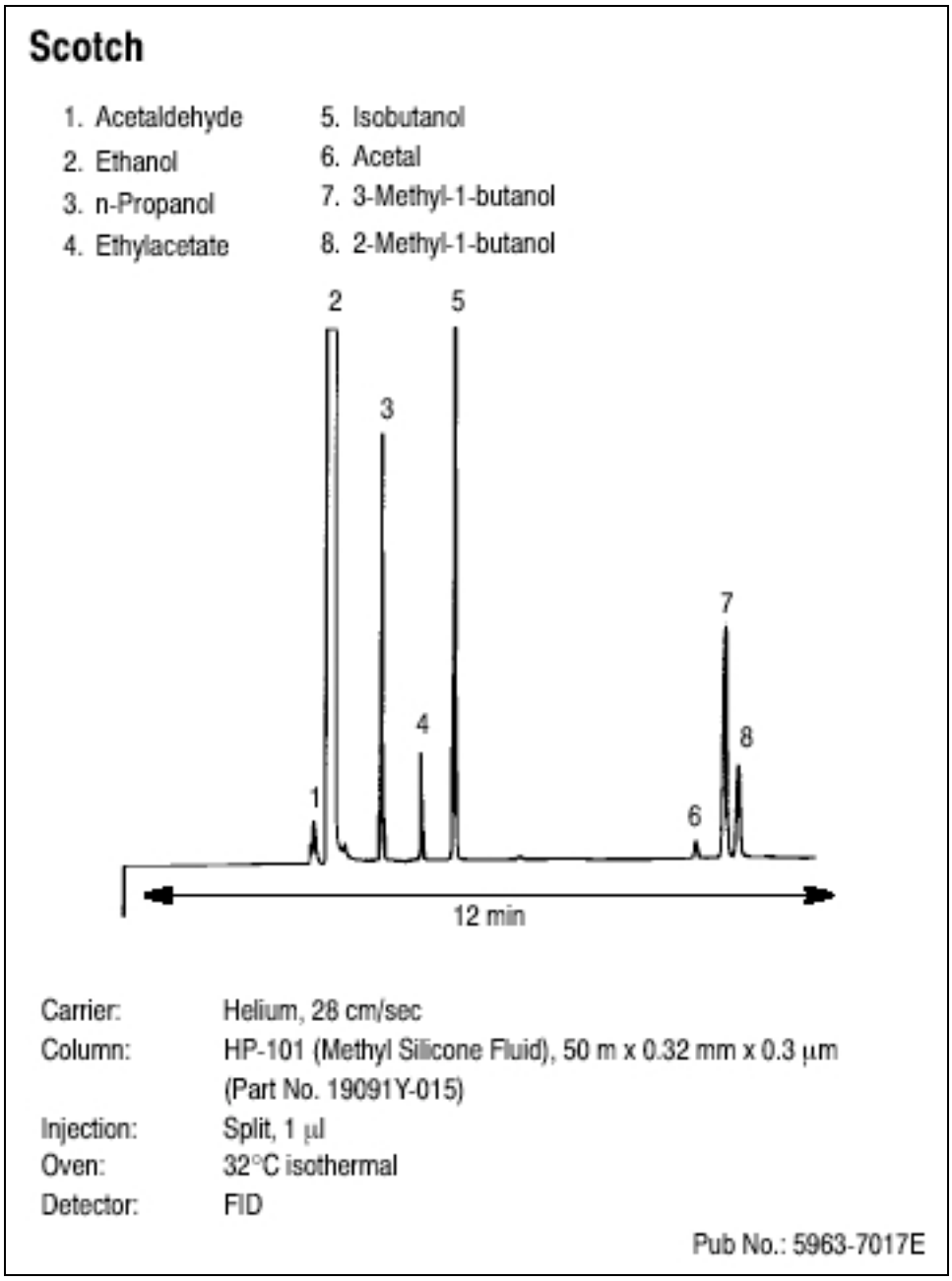


Figure 2.20 Analysis of Minor Components of Scotch by GC-FID using a HP-101 Column. Source: Copyright 2006 Agilent Technologies, Inc. Reproduced with Permission.

2.6 Summary

This chapter focused on the use of gas chromatography. A variety of separation columns and detectors allow the analysis of a diverse set of

chemical structures and the separation of complex mixtures of chemicals. Advances in technology have increased the utility of GC analysis and automated instrument controls have greatly decreased the cost of analysis by decreasing labor costs. GC allows the relatively rapid analysis of analytes present in high concentrations, such as in product quality assurance/quality control and in product identification, as well as in the analysis of trace analysis such as the identity of pollutants in environmental media and confirmation of medicines or illicit drugs in human samples. As noted several times in this chapter, GC is used to analyte volatile, thermally stable compounds, and in general cannot be used to analyze for the more extensive and diverse compounds found in biological systems. These compounds are typically analyzed for by HPLC, the subject of the next chapter. Later, in Chapter 5, GC, HPLC and CE will be coupled with MS, the ultimate detector since it allows immediate confirmatory identification. Identification by fragmentation pattern in GC analysis is the subject of Chapter 6.

2.7 Questions

1. In approximately what decade was chromatography first discovered?

2. What is the purchased price of a basic GC?

3. What are the four basic types of sample matrices?

4. List and describe the common ways gaseous samples are collected for GC analysis.

5. List and describe the common ways liquid (aqueous) samples are prepared for GC analysis.

6. List and describe the common ways soil and sediments samples are extracted and prepared for GC analysis.

7. How are biological samples prepared for GC analysis?

8. Why are analysts concerned with extraction efficiency when they prepare samples for chromatographic analysis?

9. What chemical characteristics must a chemical have in order to be analyzed by GC?

10. How are derivatizing agents used in GC analysis?

11. Draw a diagram showing all of the components of a basic GC.

12. How are the mobile phase flow rates different between packed column and capillary column GC?

13. What is the most common carrier gas used in capillary column GC?

14. Draw and explain how a split-splitless injector works. Why do we use a combination of splitless and split injection? When would you advantageous or necessary to use a total splitless injection?

15. Why is it important to use a Teflon coated septum in some GC analyses?

16. What is the purpose of the glass liner in the GC injector?

17. What are the typical sizes (diameters and column lengths) of fused silica capillary columns?

18. What are typical injection volumes for capillary column analysis?

19. Contrast the cost of an automatic sampler with the advantages of using one.

20. List the six common types of stationary phases used in GC and describe what types of analytes they can be used to analyze.

21. What are the advantages (and disadvantage) of cross linking the stationary phase coating on a capillary column.

22. List five common GC detectors, give their acronym, list the types of chemicals they are commonly used to detect, and their detection limits.

23. Explain, with drawings, how a thermo-conductivity detector (TCD) works.

24. Explain, with drawings, how a flame ionization detector (FID) works.

25. Explain, with drawings, how an electron capture detector (ECD) works.

26. What is meant by confirmatory analysis in chromatography.

27. For each of the chromatograms shown in Section 2.5, identify the intermolecular force involved in the separation of each class of analytes.

28. Select a compound that can be analyzed by GC (relatively volatile and thermally stable) and use the Internet to find what GC column and temperature program is used in its analysis.

29. Say that you are analyzing a mixture of compounds by GC and that you are having trouble achieving separation of some of them (they co-elute or appear as a shoulder peak). What three major things can you change about your GC to possible improve separation?

2.8 References:

Several instrument and column manufacturers were consulted while researching this chapter. Manufacturers have excellent web sites for researching their products, talented sales staff, and very helpful technical assistance.

Bartle K.D. and P. Myers. History of gas chromatography, Trends in Analytical Chemistry, Volume 21, Number 9, 10 September 2002, pp. 547-557